Proposed synopsis on

QUALITY BY DESIGN APPROACH FOR BIOAVAILABILITY ENHANCEMENT OF SOME HYDROPHOBIC DRUGS

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Introduction

It has been well explained that solubility, dissolution and gastrointestinal permeability are fundamental parameters that control rate and extent of drug absorption and its bioavailability. The water solubility of a drug is a fundamental property that plays an important role in the absorption of the drug after oral administration. The drug solubility is an equilibrium measure but also the dissolution rate at which the solid drug or drug from the dosage form passes into solution is critically important when the dissolution time is limited. Although the oral bioavailability of a drug depends on aqueous solubility, drug permeability, dissolution rate, first-pass metabolism and susceptibility to efflux mechanisms, aqueous solubility and drug permeability are alsoimportant parameters attributed to oral bioavailability. In drug discovery, the number of insoluble drug candidates has increased in recent years, with almost 70% of new drug candidates showing poor water solubility for these drug candidates, poor aqueous solubility and poor dissolution in the GI fluids is a limiting factor to the *in vivo* bioavailability after oral administration. Therefore, in vitro dissolution has been recognized as an important element in drug development and thus increasing the dissolution rate of poorly soluble drugs and enhancing their bioavailability is an important challenge to pharmaceutical scientists.

Solubility and dissolution are the core concepts of any physical or chemical science including biopharmaceutical and pharmacokinetic considerations in therapy of any medicine. But as the synthetic approach is growing successfully to deliver many promising lead compounds for most of the pharmacological categories, they are also taking the molecules towards bulkier structures.[01]

When an active agent given orally, it must first dissolves in gastric and/or intestinal fluids before it can permeate the membranes of the GI tract to reach systemic circulation. Therefore, a drug with poor aqueous solubility will typically exhibit dissolution rate limited absorption, and a drug with poor membrane permeability will typically exhibit permeation rate limited absorption. Hence, two areas focus on improving the oral bioavailability of active agents include:

1. Enhancing solubility and dissolution rate of poorly water-soluble drugs.
2. Enhancing permeability of poorly permeable drugs.[02]

There are various techniques available to improve the solubility of poorly soluble drugs

are Niosomes, Liquid Solid Technique, Solid dispersion, pH adjustment,Self-emulsifying drug delivery systems, Particle size reduction, Super critical fluid (SCF) process, Inclusion complexes/complexation, Co-solvency, Miceller solubilization, Polymeric Micelles, Hydrotrophy, Cryogenic techniques.

Some of the approaches to improve the solubility by nanotechnology are Nanonization, Nanocrystals, Nanosuspension, Nanoemulsion, Nanospongeand also some specific Nanotechnology approaches to improve the solubility of hydrophobic drugs are like Biorise, IDD (Insoluble Drug Delivery), CAP (Calcium Phosphate-based nanoparticles), CAP (Calcium Phosphate-based nanoparticles), NAB (Nanoparticle Albumin-Bound Technology, Nanoedge, Bio Silicon, Nano Gate, NLC8 (Nanostructured Lipid Carriers).

Quality by Design (QbD) is increasingly becoming an important and widely used technique in the pharmaceutical industry. QbD can be considered to be systems-based approach to the design, development, and delivery of any product or service to a consumer. QbD is a systematic approach to pharmaceutical development that begins with predefined objectives and emphases product and process understanding and process control. It means designing and developing formulations and manufacturing processes to ensure predefined product quality objectives. QbD identifies characteristics that are critical to quality from the perspective of patients, translates them into the attributes that the drug product should possess and establish how the critical process parameters can be varied to consistently produce a drug product with the desired characteristics. The main concept of QbD is that all final product-critical quality attributes are affected by raw materials and process parameters. Hence, if we identify the cause and effect relationship between the various inputs and responses by carefully designed experiments, we can control the quality of the product by simply controlling the inputs like raw material specifications or process parameters etc. As a result, the final product will always conform to the quality specifications.

An attempt has been made to enhance the solubility and bioavailability of poorly soluble drug i.e. hydrophobic drugs by using various techniques like Nanonization (Nanocrystals, Nanosuspension, Nanoemulsion, and Nano sponge), Niosomes, Inclusion complexes/complexation, Miceller solubilization (Polymeric Micelles), Hydrotrophy, Cryogenic techniques etc. by QbD approach.

Objectives:

Present study aimed for**, Quality by Design approach for Bioavailability enhancement of some hydrophobic drugs**, with objectives-

- To enhance the bioavailability of marketed water insoluble drug candidate in desired dosage form by QbD approach.
- To study compatibility of drug and excipients by IR, DSC and HPLC.
- To characterize formulated dosage form as per ICH guidelines.
- To identify and optimize cause and effect relationship between input and responses by *in vitro* dissolution study.
- To study stability parameters as per ICH guidelines.
- To study the bioavailability by *in vivo* evaluation study.

Need of study:

In drug discovery, the number of insoluble drug candidates has increased in recent years, with 70% of new drug candidates showing poor water solubility for these drug candidates, poor aqueous solubility and poor dissolution in the GI fluids is a limiting factor to the *in vivo* bioavailability after oral administration. Therefore, *in vitro* dissolution has been recognized as an important element in drug development and thus increasing the dissolution rate of poorly soluble drugs and enhancing their bioavailability by QbD approach is an important challenge to pharmaceutical scientists.

The approachesto increase the solubility and the available surfacearea for dissolution/bioavailability are Nanonization (Nanocrystals, Nanosuspension, Nanoemulsion, and Nano sponge), Inclusion complexes/complexation, and Miceller solubilization (Polymeric Micelles) are novel one. These approaches have more opportunity to face current challenges facing by pharmaceutical industry. So attempt has made to develop novel dosage form of some hydrophobic drugs by QbD approach.

Plan of work:

- Literature survey.
- Selection of drug, procurement of drug and excipients.
- Authentication and solubility determination of selected hydrophobic API.

- Formulation of desired dosage form by QbD approach.
- Characterizations of formulation by
  a) Solubility studies
  b) Particle size
  c) Drug loading efficiency
  d) Zeta potential
  e) DSC
  f) XRD
  g) FTIR
  h) SEM
- Evaluation of formulation

  In vitro dissolution study

  *In vivo*bioavailabilitystudy
- Compatibility and stability study as per ICH guidelines

Review of literature

Yu Guo*et al*.(2015) had worked on formulation of rebamipidenanocrystals tablets (REB-NTs) by wet-milling technique to enhance its dissolution rate and oral bioavailability. The formulation and preparation technology were screened by single factor tests with particle size and distribution as indicators. Rebamipide nanocrystals (REB-NSs) was then achieved by freeze-dry from the prepared nanosuspension which were characterized by differential scanning calorimetry (DSC) and x-ray powder diffraction (XRD), while the vitro dissolution and the plasma drug concentration of the nanocrystals tablets were investigated.[02]

Basavaraj K. Nanjwade *et al*.(2011) had worked on Design and Characterization of Nanocrystals of Lovastatin for Solubility and Dissolution Enhancement, a nanocrystals of lovastatin was formulated by using simple precipitation method without using stabilizer or surfactant and it was found that formulation at 3 mM concentration of drug with the acetone and methanol as a solvent and at proper dilution (50 times) of drug solution with water, nanocrystals with less particle size is possible with slight change in crystallinity. It has also shown that, the drug has enhanced saturation solubility, increased dissolution rate and more bioavailable in biological fluid when drug formulated by using acetone and methanol as a solvent. Whereas drug formulation with acetonitrile has large particle size, less saturation solubility and low rate of dissolution.[03]

Malihe Shahbaziniaz *et al.*(2013) had done Dissolution Rate Enhancement of Clarithromycin Using Ternary Ground Mixtures: Nanocrystal Formation, he was prepare CLA nanoparticles from a ternary ground mixture in the presence of sodium lauryl sulfate (SLS) and polyvinyl pyrrolidon (PVP) as co-grinding water-soluble compounds, in order to improve the drug dissolution rate. Different weight ratios of CLA: SLS: PVP were ground in a dry process by planetary ball mill using different grinding ball size. Following the dissolution rate study, physical properties of the best dissolved co-ground formulation was studied. The accelerated stability studies were also conducted on the co-ground formulation. This formulation formed nanocrystals with enhanced solubility after dispersing in water.[04]

K. Amighi*et al*.(2005) had worked on preparation of nifedipine nanoparticles has been achieved using high pressure homogenization. The homogenization procedure has first been optimized in regard to particle size and size distribution. Nanoparticles were characterized in terms of size, morphology and redispersion characteristics following water-removal. Saturation solubility and dissolution characteristics were investigated and compared to the un-milled commercial NIF to verify the theoretical hypothesis on the benefit of increased surface area. Crystalline state evaluation before and following particle size reduction was also conducted through differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) to denote eventual transformation to amorphous state during the homogenization process.[05]

Dr. Abdul Hasan Sathali. A. *et al*.(2013) had worked on development of nanocrystals of Paliperidone in order to enhance solubility and dissolution rate by decreasing particle size of drug and also sustained the drug release profile by using Eudragit L100 as polymer. The Paliperidone nanocrystals with small and uniform particle size were successfully prepared by nanoprecipitation method using Eudragit L100 as polymer at different ratios.[06]

Gajanan Shinde *et al.* (2015) had worked on to formulate and characterize nanocrystals formulation of Repaglinide for diabetes therapy. Formulation was done by high pressure homogenization. HPH pressure and cycles range were screened by preliminary batches (T1 and T2). 5, 8, and 10 cycles and 500 to 1500 bar pressure range had kept for further investigation. Taguchi design was used to optimize type of polymer, % polymer concentration, number of cycles, and HPH pressure for nanocrystal formulation. Formulations were characterized for particle size, zeta potential, and *in vitro* drug release.[07]

Phanchaxari M Dandagi *et al.* (2011) had worked onformulation ofgriseofulvin by Nano crystallization for the enhancement of solubility and dissolution property of drug. In the present study the area of interest are drugs belonging to class II of BCS classification. The drug nanocrystals (NC) were prepared by emulsion solvent diffusion method. Two different solvents such as acetone and ethanol and two different stabilizers such as β-cyclodextrin and sodium lauryl sulphite (SLS) were evaluated in the process.[08]

K. Masilamani*et al.* (2012)had done study the effect of polymer and surfactant concentration and sonication time/agitation speed on drug content and entrapment efficiency of nanosuspension of a water insoluble drug Aceclofenac. Nanosuspension of Aceclofenac for oral route were prepared by o/w emulsion method.[09]

Samar A. Afifi *et al*.(2015) had study on Etodolac (ET) (poorly soluble drug) nanosuspension were prepared by both pH shift method and antisolvent techniques in order to increase its dissolution rate. It was found that the antisolvent method for the preparation of ET nanosuspension reduced the drug particle size to a higher extent compared to the pH shift method. The dissolution rate of ET in distilled water was markedly enhanced in the Nano sized system, as more than 65%of drug dissolved in 10 min from all the nanosuspension formulations.[10]

Rupali L shid *et al*.(2014) had done investigation of to increase the solubility and dissolution rate of simvastatin by the preparation of nanosuspension by emulsification solvent diffusion method.[11]

Neha Tyagi *et al*.(2013) had worked on isolate a novel bio material from the seeds of Buchanania Lanzan and to evaluate their potential for sustained drug delivery by formulating various nano suspension using methylene chlorides as organic solvent and biomaterial. The biopolymer was isolated from the seeds of Buchanania Lanzan by simplified economical method. Fiveformulations were prepared using biomaterial in different ratios by solvent evaporation according to OECD guidelines. The formulated nano suspension were subjected for various evaluation parameters like particle size and shape, drug content, entrapment efficacy and % transmittance and invitro drug release studies.[12]

Satyanarayan Pattnaik (2014) had doneAceclofenac (a BCS II drug) nanosuspension were prepared using a very simple top down approach and various concentrations of surfactant were used to optimize the nanosuspension. Surfactant concentration at a level of 0.5% was found to

improve the dissolution rate of Aceclofenac due to significant decrease in particle size in the nanosuspension.[13]

Kiran Thadkala *et al*.(2016) had worked onto prepare and investigate better and stable amorphous ezetimibenanosuspension for oral bioavailability enhancement. The nanosuspension of ezetimibe was successfully prepared using solvent-antisolvent precipitation. The two factors solvent: antisolvent ratio and surfactant concentration influenced the particle size of the nanosuspension prepared. Nanosuspensions were smooth and spherical. The X-ray powdered diffraction and differential scanning Calorimetry results indicated that the anti-solvent solvent method led to the amorphization of ezetimibe. Under storage, the amorphous ezetimibe nanosuspension demonstrated significant physical stability.[14]

F.A. Araujo *et al*.(2010) had worked on development of Nanoemulsion intended for intravenous administration of thalidomide(THD). The formulations were prepared by spontaneous emulsification method and optimized with respect to thalidomide (0.01–0.05%, w/w), and hydrophilic emulsifier (polysorbate 80; 0.5–4.0%, w/w) content. The formulations were evaluated concerning physical appearance and drug crystallization; droplet size; zeta potential and drug assay.[15]

Khalid A. Ansari*et al.*(2010) had done increase the solubility, stability and permeation of resveratrol bycomplexation with cyclodextrin-based Nano sponges (NS). Nano sponges are recently developed hyper cross- linked cyclodextrin polymers nanostructured to form three-dimensional networks; they are obtained by reacting cyclodextrin with a cross-linker such as carbonyldiimidazole. They have been used to increase the solubility and stability of poorly soluble actives.[16]

Vidya Viswanad *et al.* (2015) had worked on Nano sponges of cephalexin were prepared using hydroxyl ethyl cellulose and poly vinyl alcohol by emulsion solvent evaporation method. The permeation studies showed that the formulation having higher concentration of permeation enhancer showed good skin permeation. The histological investigation on porcine skin indicated a disruption of the stratum corneum, suggesting improved permeation of the drug.[17]

Franca Castiglione *et al.* (2014) had done Ibuprofen sodium salt (IP) was encapsulated in cyclodextrin Nano sponges (CDNS) obtained by cross-linking of β-cyclodextrin withethylenediaminetetraacetic acid dianhydride (EDTAn) in two different preparations: CDNSEDTA 1:4 and 1:8, where the 1: *n* notation indicates the CD to EDTAn molar ratio. The

entrapment of IP was achieved by swelling the two polymers with a 0.27 M solution of IP in D2O, leading to colorless, homogeneous hydrogels loaded with IP. The molecular environment and the transport properties of IP in the hydrogels were studied by high resolution magic angle spinning (HRMAS) NMR spectroscopy.[18]

Gautam Seema *et al*.(2015) had done development and evaluation of curcumin loaded Nano sponges for colon drug delivery. Development of curcumin loaded Nano sponge will increase the bioavailability and retention time at the colon. Due to small size and porous nature of the novel delivery system they can easily bind to poorly-soluble drugs within their matrix and prevent drug from rapid metabolism and excretion, improve their bioavailability. The Nano sponge of curcumin is prepared by solvent evaporation method. Further the formulated Nano sponges are evaluated for its Phase Solubility, Particle Size Determination, Zeta Potential, SEM, TEM analysis, Loading Efficiency, and Production Yield and in vitro release study.[19]

CH.N.V. Raja *et al.* (2013) had done the formulated Nano sponge loaded with Ciprofloxacin antibiotic used resulted in sustained release.The drug is acid labile and hence it is entrapped with ethyl cellulose for its sustained release. As the drug made into nanoparticle the density was found to be increased. Nano sponge is water soluble.[20]

Dr. Prathima Srinivas *et al*.(2013)had done to produce controlledrelease VoriconazoleNano sponges for topical and oral delivery. Nano sponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared successfully using PVA as surfactant by emulsion solvent evaporation method. The effects of different drug: polymer ratios, surfactant concentration, stirring speeds and time, sonication time on the physical characteristics of the Nano sponges as well as the drug entrapment efficiency of the Nano sponges were investigated. Particle size analysis and surface morphology of Nano sponges were performed. The optimized Nano spongeformulations were selected for preparing Nano spongetablets for controlled drug delivery by oral route.[21]

Gouri Shankar *et al*. (2015) had doneto develop Simvastatin loaded β-cyclodextrin Nano sponges with carbonyldiimidazole as across linker and chitosan as mucoadhesive agent for oral drug delivery for enhancing the solubility of Simvastatin so that the bioavailability problems are solved. Solid dispersion technique has been used for drug incorporation.[22]

Ashwini Deshpande *et al*. (2014) had donecyclodextrin-based Nano sponges of atorvastatin were prepared by condensation polymerizationand interfacial polymerization to release

Atorvastatin in expected manner in the treatment of dyslipidemia as novel carriers. Results of encapsulation efficiencies of all formulation trials revealed that condensation polymerization is the best method fornanosponges' formation and that is considered as best selected method for preparation.[23]

Patil Bhagyashree *et al*.(2016) had worked onto produce controlled release ArtesunateNano sponges for topical and oral delivery. Nano sponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared using PVA as surfactant by emulsion solvent evaporation method. The effects of different drug: polymer ratios, surfactant concentration, stirring speeds and time, sonication time on the physical characteristics of the Nano sponges as well as the drug entrapment efficiency of the Nano sponges were investigated.[24]

Maria Carafa *et al*.(2013) study the A new delivery system based on ibuprofen-βcyclodextrin (βCd) complexation and its loading into non–ionicsurfactant vesicles (NSVs) was developed to improve ibuprofen therapeutic efficacy in topical formulations. The proposed strategy exploits the well-known solubilizing and stabilizing properties of cyclodextrins together with the high tolerability and percutaneous absorption enhancing properties of NSVs. The complexing capacity of Cds in the presence of Ibuprofen in aqueous solution was evaluated by means of phase solubility studies. The technique used to obtain solid ibuprofen-βCd complexes was the co-lyophilization method.[25]

Biswajit Mukherjee *et al*.(2007) worked on designed to develop and compare acyclovir containingnano-vesicular liposomes and niosomes based on cholesterol, soya L-α-lecithin and nonionic surfactant, span 20. The effort was made to study in vitro whether acyclovir-loaded Nano vesiclescould sustain the release of the drug by increasing residence time and thus, acyclovir could reduce its dose-related systemic toxicity. There were good vesicular distributions in both of the niosomes and the liposomes.[26]

Deepak Kumbhar *et al*.(2013) had done Niosomal gel of lornoxicam was developed for topical application.Lornoxicam niosomes (Lor-Nio) were fabricated by thin film hydration technique. Bilayer composition of niosomal vesicles was optimized. Lor-Nio dispersion was characterized by DSC, XRD, and FTIR. Morphological evaluation was performed by scanning electron microscopy (SEM).[27]

Elsie Oommen*et al.* (1999) had done to entrap methotrexate (MTX) complexes with β-Cyclodextrin (βCD) into niosomes, tocharacterize niosomes for different physicochemical properties and to investigate the potential of niosomesentrapped MTX- β-cyclodextrin complex in tumor treatment.[28]

Vijay S. Jatav *et al.*(2011) had doneto prepare niosomes containing rifampicin using various nonionic surfactants ofsorbitan ester class and cholesterol in 50:60 (1:1.2) percent mol fraction ratios for sustain release. To improve the dissolution rate of noisome are prepare handshaking method using Surfactants and cholesterol (150 μmol) in 50:60 (1:1.2) percent mol fraction ratio.[29]

Sushama Talegaonkar *et al*.(2015) had done Design and development of novel bioadhesive Niosomal formulation for the Trans corneal delivery of anti-infective agent: In-vitro and ex-vivo Investigations. A chitosan coated niosomal formulation of gatifloxain was purposed to show a longer retention time on eyes and subsequent reduction in dosing frequency. Vesicles were prepared by solvent injection method using cholesterol and Span-60.[30]

VedhaHariB.N.*et al*.(2011) had studyto prepare Orlistat niosomesfrom proniosomes to improve its poor and variable oral bioavailability. The non-ionic surfactant vesicles are prepared by the reverse phase evaporation technique (slurry method).[31]

Payal H. Patil *et al*. (2013) had done enhance the solubility and dissolution of practically water-insoluble drug raloxifene HCl (RLX), for the same two approaches that were used. In the first approach, drug was kneaded with hydroxyl propyl-$\beta\beta$-cyclodextrin (HP$\beta\beta$CD), and in the second one drug was cogrinded with modified guar gum (MGG). The drug-cyclodextrin complex andDrug-MGG cogrind mixtures were characterized by differential scanning calorimetry; X-ray diffraction studies, scanning electron microscopy, and Fourier transform infrared spectroscopy. It was concluded that the prepared inclusion complex showed a remarkable increase in solubility and dissolution of poorly water-soluble drug raloxifene.[32]

Samip S Shah *et al*.(2012) had doneinclusion complexes of ITZ andhydroxyl propyl β-cyclodextrin (HPβCD) were prepared to enhance solubility and in vitro dissolution rate of the drug. Inclusion complex in solution was studied by phase solubility technique.[33]

Sarasija Suresh *et al*.(2009) had done the inclusion complexes of rifampicin with sucralose and β-cyclodextrins were prepared by spray drying method.The complexes were characterized by

size analyses, scanning electron microscopy, differential scanning Calorimetry and x-ray diffraction methods.[34]

Xiao-Bing Xiong *et al.* (2008) had done to develop multifunctional RGD-decorated poly(ethylene oxide)-b-poly(ester) based micellesand assess their pH-triggered core degradation and targeted drug release in tumor cells that overexpress RGD receptors.[35]

Imran Shekh *et al*.(2011) had done study the influence of β-cyclodextrin (β-CD) on the biopharmaceutical properties of aspirin. To this purpose the physicochemical characterization of aspirin-β-cyclodextrin binary systems was performed both in solution and solid state.[36]

Pushpa Rajagopalan *et al*.(2013) had worked onCyclodextrins and modified Cyclodextrins are used as complexing agents to increase the aqueous solubility, bioavailability and stability of the drugs. Inclusion complex formation of aspirin with hydroxy propyl β- Cyclodextrin (HPβ-CD) resulted in stability and solubility enhancement of the drug. Complexation was proved by UV-Vis, FT-IR, 1H NMR and XRD studies.[37]

Marcia Silva *et al*.(2007) had donethe characterization of polymeric micelle-forming tuberculostaticprodrugs and the antimycobacterial activity of this prodrugs.[38]

Nilufer Yaksel *et al*.(2006) had done to investigate the solubilization of poorly water-soluble anticancer drugs, octaethylporphine (OEP),meso-tetraphenyl porphine (mTPP) and camptothecin (CPT), in Pluronic and polyethylene glycol–distearoylphosphatidylethanolamine (PEG–DSPE) polymeric micelles. Three different Pluronic and PEG–DSPE polymers with various chain lengths were chosen and micelle formulations were prepared by using various drug: polymer ratios. Formulations were characterized by critical micellization concentration(CMC) values of copolymers, micelle particle size and distribution, zeta potential, loading efficiency and stability.[39]

Yoshiyuki Hattori *et al*.(2016) had done prepared PM and hybrid nanoparticles (HNPs) with poly(ethylene oxide)-block-poly(methacrylicacid) (PEO-b-PMAA) for loading cisplatin, and evaluated cisplatin release, cytotoxicity, and bio distributionin mice. PM composed of PEO-b-PMAA and HNPs composed of egg phosphatidylcholine (EPC)/PEO-b-PMAA at molar ratios of 50/2.8 (HNP-P5) and 50/50 (HNP-P50), respectively, were prepared by a nanoprecipitation method.[40]

Xi Yang *et al*.(2015) had worked on To develop injectable formulation and improve the stability of curcumin (Cur), Cur was encapsulatedinto monomethyl poly (ethylene glycol)-poly (ε-

caprolactone)-poly (trimethylene carbonate) (MPEG-P(CL-*co*-TMC)) micelles through a single-step solid dispersion method.[41]

Graciette Matioli *et al*.(2013) had done Curcumin was complexes with b-CD using co-precipitation, freeze-drying and solvent evaporation methods.Co-precipitation enabled complex formation, as indicated by the FT-IR and FT-Raman techniques via the shifts in the peaks that were assigned to the aromatic rings of curcumin.[42]

K. Sivakumar *et al*.(2015) had worked on Host–guest inclusion complexation of acetanilide (AA) into the β-cyclodextrin (β-CD) nano-hydrophobic cavity has beeninvestigated using computational technique. Two different orientation of guest (AA) into host (β-CD) are considered for the inclusion of AA in to the Nano cavity through the 2o rim of β-CD. The energy differences in the inclusion process are calculated using PM3 semi-empirical method.[43]

Abdalla Ahmed Elbashir *et al*.(2013) had donethe host–guest inclusion complex between β-cyclodextrin (β-CD) and cephalexin was investigated spectrofluorometrically.[44]

M. J. Ansari *et al.*(2015)had doneto evaluate solubility and dissolution of ciprofloxacin using current nanotechnologyand classical cyclodextrin inclusion complex approach. Ciprofloxacin nanosuspension was prepared by anti-solvent precipitation method using pluronic F68 as stabilizer. Phase solubility study of ciprofloxacin was carried out alone and with addition of different amount of beta-cyclodextrin.[45]

Kaiyan Lou *et al*.(2016) had done a novel adamantanamine-paclitaxel (AD-PTX) incorporated oligochitosancarboxymethyl-β-cyclodextrin (CSO-g-CM-β-CD) self-assembly macromolecular (CSO-g- CM-β-CD@AD-PTX) micelle was successfully prepared in water through sonication. The formed molecules were characterized by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance (NMR) spectroscopy, two-dimensional NMR, elemental analysis, and liquid chromatography-mass spectrometry, while the correspondent micelles were characterized by dynamic light scattering and transmission electron microscopy.[46]

Materials and Methods:

1. Methods for preparation of Nano sponges

a) Nanosponges prepared from hyper-cross linked β-cyclodextrin

100 mL of anhydrous dimethylformamide (DMF) were placed in a round bottom flask and 17.42 g of anhydrous β-cyclodextrin (15.34 mmol) were added to achieve complete

dissolution. Then 9.96 g of carbonyldiimidazole(61.42 mmol) were added and the solution allowed reacting for 4 h at 100◦C. Once the condensation polymerization was completed, the transparent block of hyper-cross-linked cyclodextrin was roughly ground and an excess of deionized water added to remove DMF. Finally, residual by-products or unreacted reagents were completely removed by Soxhlet extraction with ethanol. The white powder thus obtained was dried overnight in an oven at60°C and subsequently ground in a mortar. The fine powder obtained was dispersed in water. The colloidal part that remained suspended in water was recovered and lyophilized. The nanosponges recovered are sub-micron in dimension and with a spherical shape. The cyclodextrin: cross-linker molar ratio can vary (i.e. 1:2, 1:4, 1:8). Nanosponges can be classified according to the molar ratio with the cross-linker used in their preparation (i.e. nanosponges, 1:4).[16]

b) Emulsion solvent diffusion method

In this method 2 phases are used in different proportion of organic and aqueous(ethyl cellulose and polyvinyl alcohol). The dispersed phase having ethyl cellulose and drug get dissolved in dichloromethane(20 ml) and a definite amount of polyvinyl alcohol added to 150 ml of aqueous continuous phase. Then, the mixture is stirred properly at 1000 rpm for 2hr. The required nanosponges were collected by the process of filtration and kept for drying in oven at 40ºc for 24hr. Nanosponges which are dried were stored in desiccators and ensured of removal of residual solvents is done.[24]

c) Quasi-emulsion solvent diffusion

The nanosponges prepared using the polymer in different amounts. The inner phase is prepared using Eudragit RS 100 and added to a suitable solvent. Drug used provided with a solution and dissolved under ultra-sonication at 35ºc. This inner phase added into external phase containing pva act as emulsifying agent. The mixture is stirred at 1000-2000 rpm for 3hr at room temperature and dried in an air-heated oven at 40ºc for 12hr.[24]

2. Methods for preparation of Niosomes:

a) Preparation of niosomes by freeze drying method

A suspension of drug inthe βCd solution in Hepes buffer (10 mM pH 7.4) was prepared.

The suspension was shaken at 400 rpm on a mechanical stirrerthermo stated at a temperature of 50°C for 2 days and then filtered to remove the excess of drug. The resulting solution in glass

vials was frozen at -80°C and then was freeze dried. Physical mixtures (PM) of drug and βCd were prepared by mixing in a mortar.[25]

b) Preparation of niosomes by hand shaking method

In this the liquid mixture was dissolved in 10ml diethyl ether in a roundbottom flask. The solvent was evaporated using a rotary evaporator until complete evaporation of solvent was ensured and the surfactant film deposited on the wall of the flask was hydrated with 5ml of phosphate buffer saline (PBS, pH 7.4) containing drug (10mg/ml) at 60±2oC for 1h to obtain niosomal dispersion.

Surfactants andcholesterol (150 μmol) in 50:60 (1:1.2) percent mol fraction ratios were dissolved in 10 ml diethyl ether in a 50 ml round-bottom flask, and ether was removed at an ambient temperature (37±1°C) under reduced pressure in a rotary flash evaporator. The dried film of surfactant was hydrated with occasional shaking for 15 min at 70°C on water bath with 5ml aqueous phase (phosphate buffer saline, pH 7.4) containing 25 mg drug. Thissuspension was then sonicated to form unilamellar niosomes.

The resultant aqueous dispersions of drug -bearing niosomes were dialyzed exhaustively inCuprophane dialysis tubing against phosphate buffer saline (pH 7.4) to separate the unentrappeddrug from the niosome-entrapped drug.[29]

3. Methods for preparation of Polymeric Micelles[36]

a. Direct dissolution technique: Direct dissolution of the amphiphilic co-polymerand drug in water is the simplest technique ofpreparing drug-loaded polymeric micelles. At or above CMC, the co-polymer and the drug self assemblein water to form drug-loaded micelles.

b. Solvent evaporation or solution-casting technique: a volatile organic solvent is used todissolve the co-polymer and the drug. A thin filmof co-polymer and drug is obtained after thesolvent is removed by evaporation. Drug-loadedpolymeric micelles are obtained by reconstitutionof film with water.

c. Dialysis technique: Solutions of the drug and the polymer in organic solvent are placed in the dialysis bag, and the solvent is exchanged with water by immersing bag into water, inducing micelle assembly.

4. Methods for preparation of Cyclodextrin Inclusion Complexes: [25]

a. Co-precipitation: CD is dissolved in water and the guest is added while stirring the CD solution.By heating, more CD can be dissolved (20%) if the guest can tolerate the higher

temperature.The CD and guest solution must be cooled under stirring before a precipitate isformed. The precipitate can be collected by decanting, centrifugation or filtration and washed.

b. **Slurry complexation:**CD can be added to water, as much as 50–60% solids, and stirred.The aqueous phase will be saturated with CD in solution. Guest molecule will complex withthe CD in solution and, as the CD complex saturates the water phase; the complex will crystallizeor precipitate.
c. **Damp mixing / Fusion method:**The guest and CD are thoroughly mixed and placed in asealed container with a small amount of water. The contents are heated to about 100 °C andthen removed and dried.
d. **Dry / Physical mixing:** Some guests can be complexed by simply adding the guest to the CD and mixing/triturating them together. This works best with oils or liquid guests.
e. **Lyophilization:** CD and the guest are mixed in de-ionized water with continuous agitation to form a solution. The solution is then frozen below 0 0C and next lyophilized using freeze drier. The dried powder is then sieved to get the complex powder.
f. **Kneading method:** The guest is dissolved in suitable vehicle at room temperature to which the required quantity of CD is added. The thick slurry is then kneaded for about 1 hour in mortar. The paste is dried in hot air oven and pulverized and sieved to get the complex powder.
g. **Spray drying:** In this method the guest and cyclodextrin both are dissolved in suitable vehicle and spray dried in spray dryers.
h. **Freeze-drying method:** In this method guest is added to aqueous solution of cyclodextrin while mixing with a magnetic stirrer. After 24 hours of agitation, the resulting solution is frozen by keeping it in a repository at 50 to 60°C and is lyophilized in a freeze-dryer for 24 hours.
i. **Solvent evaporation method:** A solution of poorly water soluble guest in suitable organic solvent is added to aqueous solution of CD and agitated at about 40 to 50 0C for 30 min and toward the end of addition turbidity will develop in the mixture, at the end of this period the solution is filtered, and the moist solid is kept in air/oven for removal of last trace of solvent.

Duration pattern for Plan of work

Sr. No.	Duration of work	Plan of work	Total months
1.	July 2016-Dec. 2016	Review of literature, selection and procurement of drug, authentication of drug.	06
2.	Jan. 2017-June 2017	Formulation by QbD approach, dependent variable and independent variable interaction study, trial basis.	06
3.	July 2017- Dec. 2017	Optimization of formulation (DOE), compatibility study.	06
4.	Jan.2018-June 2018	Characterization of formulation by DSC, XRD, FTIR, SEM, solubility study, particle size, zeta potential, drug loading efficiency.	06
5.	July 2018-Dec.2018	*In vivo* study	06

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